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# Liming and nitrogen fertilization affects phosphatase activities, microbial biomass and mycorrhizal colonisation in upland grassland

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**Abstract**

We have studied the effects of factorial combinations of lime and N additions on soil microbial biomass, respiration rates and phosphatase activity of an upland grassland. We also used an *Agrostis capillaris* seedling bioassay to assess the effect of the treatments on the activity of arbuscular-mycorrhizal (AM) fungi and root surface phosphatase enzymes and the concentrations of N and P in the bioassay plant shoots. In the F and H horizons, soil microbial biomass carbon ($C_{mic}$) decreased in response to the liming, while addition of lime and N together reduced basal respiration rates. In the Ah horizon, $C_{mic}$ was unaffected by the treatments but basal respiration rates decreased in the plots receiving nitrogen. Soil phosphatase activity decreased only in the Ah horizon in plots receiving lime, either in combination with N or alone. The mass of root fwt. colonized by AM fungi increased in response to the treatments in the order nitrogen < lime < N plus lime. In contrast, root surface phosphatase activity decreased only in response to additions of nitrogen. A positive linear relationship was observed between root surface phosphatase activity and the P concentration of the plant shoots ($R^2 = 28.7\%$, $P = 0.004$). The results demonstrate the sensitivity of both free-living heterotrophic microorganisms and symbiotic mycorrhizal fungi to short-term (2 years) applications of lime and N to long-term upland grassland, particularly in relation to the key P cycling activities undertaken by these organisms.

## Introduction

Soil microorganisms have a critical role in low-input ecosystems, such as upland grasslands, where they regulate litter decomposition and are responsible for mineralization of nutrients, particularly N and P. In low nutrient input grasslands, arbuscular-mycorrhizal (AM) fungi are major constituents of the microbial biomass. They readily colonize the roots of virtually all plant species that inhabit upland grasslands (Sparling and Tinker, 1978) and are a key group of microorganisms in ecosystems as they provide their host plants with increased amounts of nutrients, particularly P (Smith and Read, 1997). The soils of much upland grassland in the UK are characteristically rich in organic matter and have low pH and little inorganic N and P. In contrast, concentrations of organic N and P in soil are typically very high, and can account for 30–80% of the total (Dalal, 1977; Tarafdar and Claassen, 1988). In nutrient poor grassland, Macklon et al. (1994) demonstrated that orthophosphate in soil solution was insufficient for satisfying plant P demand, and suggested that soluble organic forms are likely to be important in these soils. The organisms and processes responsible for

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accessing organic P therefore have a critical role in ecosystems like upland grassland that have large organic and small inorganic P pools.

Organic P is mineralized to inorganic P by the action of phosphatase enzymes, a broad group that are of microbial or plant origin. They are found in soil solution or bound onto clay minerals and the surface of plant roots (Tabatabai and Bremner, 1969). Phosphatase enzymes bound on the surface of roots may originate from mycorrhizal or saprotrophic fungi, bacteria or root exudates. The importance of root surface phosphatases in maintaining an adequate supply of P for the plant can be considerable; inorganic P produced by this mechanism has been calculated to contribute 65% of the annual P demand of *Eriophorum vaginatum* subsp. *spissum* plants (Kroehler and Linkins, 1988). Macklon et al. (1997) demonstrated a close-coupling between plant uptake of soil P and release of extra-cellular root surface phosphatases. They showed that both shoot and root P concentrations of the grass *Agrostis capillaris* increased when the roots were immersed in a solution containing $^{32}P$ labelled organic P. In addition, the amount of dissolved organic P in the growth solution decreased and the activity of root surface phosphatases increased in proportion to the amount of P taken up by the plant. Expression of phosphatase activity may therefore not only reflect P demand but is also of considerable importance for P turnover.

Addition of lime and fertilizer (often nitrogen), undertaken to improve the productivity of soils with a low inorganic nutrient status for pastoral agriculture, has been shown to affect total soil microbial biomass (Hopkins, 1997). In addition, stimulation of soil phosphatase activity per unit microbial biomass carbon in response to atmospheric N deposition suggests that the key nutrient cycling activities performed by the soil microbial community can be highly sensitive to increased loadings of N (Johnson et al., 1998). However, the response of specific microbial groups, and in particular AM fungi, to agricultural improvement is less clear. Application of lime has been found to reduce the number of spores in a grassland in Sweden (Raznikiewicz et al., 1994), while in contrast, liming increased AM colonization of barley (*Hordeum vulgare* L.) roots in a recently cultivated meadow (Hamel et al., 1996). N fertilization has been shown to cause shifts in the AM fungal community of sage scrub (Egerton-Warburton and Allen, 2000) and also stimulate both root colonization and external mycelial development in tallgrass prairie (Eom et al., 1999), possibly as a consequence of the increased P demand under these treatments.

Seedling bioassays have been used successfully in several studies to investigate the impacts of long-term simulated N deposition on the inoculum potential of AM fungi, root surface phosphatase activities and nutrient availability in grassland (Johnson et al., 1999; Phoenix et al., 2003). This approach was adopted in the present study to determine the effects of factorial combinations of N and lime on the activity and nutritional function of AM fungi and the root surface phosphatase activity using *Agrostis capillaris* as the bioassay species. We also determined the size of the microbial biomass and the activity of soil phosphatase activities.

## Materials and methods

### *Study site*

The study was undertaken using turfs (40 cm × 25 cm × 20 cm deep) removed from an upland grassland (the NERC Soil Biodiversity field site, Sourhope, Kelso, Scotland; NGR: NT 854196; altitude: 500 m a.s.l.). Twenty turfs were removed from experimental plots (20 m × 12 m) arranged in a 5 block × 4 treatment completely randomised design comprising control, lime, N plus lime + N treatments. The lime ($CaCO_3$; 39% Ca; 0.5% ash soluble in HCl applied at a rate of 0.6 kg $m^{-2}$) and N ($NH_4NO_3$ applied at a rate of 12 g $m^{-2}$) were manually spread onto the plots as solids during April/May of each year. The whole site was mown on six occasions during the summer and all clippings were removed. The turfs were maintained outdoors at the University of Sheffield Experimental Gardens for the duration of the experiment.

The plant community comprises a mixture of 24 higher-plant species, 21 of which typically form AM-mycorrhizas (Harley and Harley, 1987), and is classified as U4d (*Festuca-Agrostis-Galium-Luzula multiflora-Rhytidiadelphus loreus* sub-community) under the British National Vegetation Classification (Rodwell, 1992). The site

has been under pasture and has received no fertiliser additions for the past 40 years. It was fenced and excluded from grazing from April 1999 onwards. The soils (Table 1) are developed on locally derived drift from andesitic lavas of Old Red Sandstone Age and are acid brown earths (pH 4.5–5.0) with a mean clay content in the 2 mm fraction of 40%.

*Seedling bioassay to assess arbuscular mycorrhizal colonization*

Seeds of *Agrostis capillaris* L. (Common bent grass), obtained from Cressbrookdale in Derbyshire, UK, were sterilised for 15 min in a saturated solution of $CaCl_2O_2$, washed with sterile distilled water and germinated on autoclaved Bunter sand. We transferred 5 seedlings 1-week after radicle emergence into the intact turfs for 10 days. The roots of the seedlings were inserted into small holes made with a pair of straight forceps. The seedlings were removed at an early stage by carefully teasing back the surrounding soil and vegetation. This was done after 10 days because (1) AMF colonisation and expression of root-surface phosphatase activity responds rapidly to perturbations (Johnson et al. 1999; Read and Birch, 1988) and (2) root extraction would be extremely difficult if the plants became too big. The roots were excised and the shoots oven-dried and weighed. The entire shoot samples (up to 7 mg) were digested in 0.5 mL of a salicylic/sulphuric acid-lithium sulphate mix for 5 h at 350 °C (Bremner and Mulvaney, 1982), diluted to 5 mL and analysed for total N (Scheiner, 1976) and P (John, 1970). The roots were washed and immediately assayed for phosphomonoesterase activity (see below). They were then cleared (10% KOH at 80 °C for 2 h), acidified (10% HCl), stained for 2 h (trypan blue in lactoglycerol), de-stained (50% glycerol) and scored for mycorrhizal colonization at ×300 magnification (McGonigle et al., 1990). The data were expressed both as percent root length colonized and the amount root fresh weight colonized.

*Basal respiration, soil microbial biomass and enzyme activities*

We measured respiration rates of soil microbial communities using a sealed system 'Respicond' (Nordgren Innovations AB., Umeå) automated conductometric respirometer (Nordgren et al. 1988). Intact 4 cm diameter soil cores were removed from each turf, separated into the FH and Ah horizons and sieved (2 mm). A 10 g subsample was incubated at 22 °C and respiration rates logged every 30 min for 8 h. Microbial biomass C ($C_{mic}$) was determined by substrate induced respiration (Anderson and Domsch, 1978). We incubated a 10 g soil sample at 22 °C after adding glucose (using a previously optimized concentration of 10 mg g $dwt^{-1}$ added in 2 mL water), and respiration rates were measured every 20 min. The maximum rate measured within the initial 4 h of incubation was used to calculate $C_{mic}$ (Anderson and Domsch, 1978).

*Table 1.* Soil pH (water) and concentrations of $NaHCO_3$-extractable P from the FH and Ah horizons (±SE) and *P*-values derived from ANOVA of N and lime effects

| Treatment | PH | | Extractable P (mg $g^{-1}$ dwt) | |
|---|---|---|---|---|
| | FH | Ah | FH | Ah |
| Control | $5.3^{c}$ ± 0.09 | 5.0 ± 0.06 | 178 ± 41 | 8.1 ± 1.8 |
| N | $5.7^{b}$ ± 0.16 | 5.4 ± 0.13 | 162 ± 40 | 6.3 ± 0.6 |
| Lime | $8.0^{a}$ ± 0.04 | 5.9 ± 0.18 | 206 ± 23 | 12.5 ± 2.4 |
| N plus lime | $7.8^{a}$ ± 0.08 | 5.9 ± 0.17 | 222 ± 22 | 13.2 ± 2.9 |
| *P*-values from ANOVA | | | | |
| N | 0.193 | 0.139 | 0.197 | 0.016 |
| Lime | 0.000 | 0.000 | 0.997 | 0.703 |
| N plus lime | 0.008 | 0.136 | 0.635 | 0.657 |

Values sharing a letter (N plus lime effects only) are not significantly different ($P > 0.05$; $n = 5$)

We calculated the metabolic quotient ($qCO_2$) by dividing basal respiration by $C_{mic}$ (Anderson and Domsch, 1993).

A 0.5 g subsample was used for assay of phosphomonoesterase (PMEase) activity using the artificial substrate *p*-nitrophenyl phosphate (*p*-NPP; Tabatabai and Bremner, 1969). 1 mL of soil suspension (1 g soil to 14 mL water) and 4 mL of 13.6 m*M* *p*-NPP solution were added to universal vials and assayed for 45 min at 37 °C in a shaking water bath. The solutions were centrifuged (3000 rpm) and a subsample (100–500 $\mu$L) added to 2 mL 2 *M* NaOH and the optical density measured immediately using a Cecil CE1020 spectrophotometer at a wavelength of 410 nm. The assays were run without buffer in order to allow the enzyme to operate at the pH of the soil. The enzyme activity was quantified against a set of standards prepared in the range 0–80 n*M*. Determination of root surface PMEase activities followed a similar procedure, except that only 2 mL of substrate was used and this was buffered with 0.2 *M* TRIS/maleate buffer. The assay was buffered to pH 5.5 for plants grown in the control and N treated plots, and to 7.8 for plants grown in the plots treated with lime and N plus lime.

## Results

In general, the lime and N treatments had a significant effect on soil pH and extractable P concentrations (Table 1). In the FH horizon, soil pH in the control was 5.3 and this increased significantly ($P = 0.008$) to 8 in the lime treatment and 7.8 in the lime plus N treatment. A more modest increase to 5.7 was recorded for the N treatment. In the Ah horizon, the overriding influence was lime, which increased ($P < 0.001$) soil pH from 5.2 in the control and N treatments to 5.9 in the lime and lime plus N treatments.

Extractable soil P concentrations in the FH horizons did not differ significantly between the treatments but ranged from 162 mg g dwt$^{-1}$ in the N treatment to 222 mg g dwt$^{-1}$ in the lime plus N treatment. In contrast, in the Ah horizon, the concentrations increased in response to N inputs ($P = 0.016$). In control and lime treatments, the concentrations were 8.1 and 6.3 mg g dwt$^{-1}$ respectively whilst in the lime and lime plus N treatments they increased to 12.5 and 13.2 mg g dwt$^{-1}$ respectively.

Colonization of *A. capillaris* by AM fungi ranged from 13 to 37% of the root length (Figure 1a). The greatest values were in the plots receiving lime and lime and N, where AM colonization was 30 and 37% respectively, but these were not significantly different from the control plants. The mass of root fresh weight colonized ranged from 140 to 600 $\mu$g (Figure 1b), the values increasing linearly in the treatment order control < N < lime < lime plus N. A significantly greater ($P < 0.05$) amount of root was colonized by AM fungi in plots receiving lime, either alone or in combination with N (Figure 1b).

The shoot dry weight of the bioassay seedlings ranged from 0.85 to 2.56 mg (Table 2). The greatest values were recorded in the N and N plus lime plots, these being significantly different from the plants grown in the control and lime plots ($P < 0.05$). Shoot N concentrations ranged from

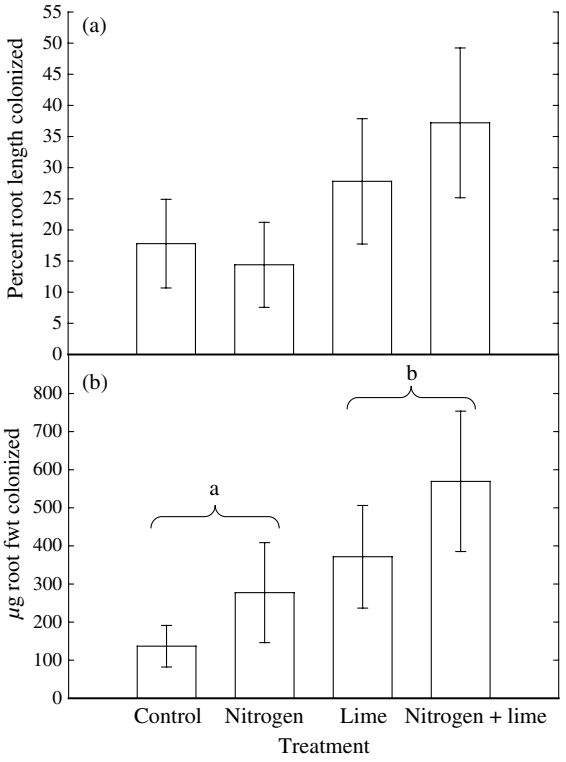


*Figure 1.* Colonization by AM fungi of 10-day-old *A. capillaris* bioassay seedlings as (a) percentage of root length and (b) as $\mu$g root fwt$^{-1}$ ($\pm$SE). Letters indicate an overall liming effect ($P = 0.044$; $n = 5$).

*Table 2*. Shoot dwt, concentration and total shoot N and P and N:P ratio in bioassay seedlings of *A. capillaris* (±SE) and *P*-values derived from ANOVA of N and lime effects

| Treatment | Shoot dwt (mg) | Shoot N (mg g dwt$^{-1}$) | Shoot P (mg g dwt$^{-1}$) | Total N (mg) | Total P (mg) | Shoot N:P |
|---|---|---|---|---|---|---|
| Control | 0.85 ± 0.26 | 29.0$^{ab}$ ± 2.0 | 13.6 ± 3.0 | 43.2 ± 14.8 | 15.5 ± 2.1 | 2.8 ± 0.9 |
| N | 2.41 ± 0.56 | 25.9$^{b}$ ± 2.1 | 6.4 ± 1.1 | 84.6 ± 18.6 | 22.4 ± 4.0 | 4.8 ± 1.1 |
| Lime | 1.12 ± 0.29 | 26.9$^{ab}$ ± 3.1 | 15.1 ± 3.1 | 59.0 ± 19.4 | 32.1 ± 4.8 | 2.1 ± 0.5 |
| N plus lime | 2.56 ± 0.41 | 34.3$^{a}$ ± 1.6 | 10.4 ± 1.1 | 92.8 ± 13.7 | 26.2 ± 3.3 | 3.7 ± 0.4 |
| *P*-values from ANOVA | | | | | | |
| N | 0.359 | 0.176 | 0.082 | 0.503 | 0.027 | 0.222 |
| Lime | 0.000 | 0.348 | 0.007 | 0.043 | 0.919 | 0.021 |
| N plus lime | 0.975 | 0.027 | 0.355 | 0.853 | 0.138 | 0.752 |

Values sharing a latter (N plus lime effects only) are not significantly diffenent ($P < 0.05$; $n = 5$)

25.9 mg g$^{-1}$ dwt in the N treated plots to 34.3 mg g$^{-1}$ dwt in the N plus lime treated plots, these values being significantly different. This pattern was not reflected in the total shoot N contents. These were 43.2 and 59 mg in the control and lime plots respectively. These increased significantly to 84.6 and 92.8 mg in the N and N plus lime treated plots, respectively. Shoot P concentrations were 6.4 and 10.4 mg g$^{-1}$ dwt in the N and N plus lime treated plots respectively (Table 2). They increased significantly to 13.6 mg g$^{-1}$ dwt in the control and to 15.1 mg g$^{-1}$ dwt in the plots treated with lime. In contrast, total shoot P contents were significantly greater in plots receiving lime and N plus lime (Table 2). In these treatments, the total shoot P contents were 32.1 and 26.2 mg, respectively, and in the control and N treated plots 15.5 and 22.4 mg, respectively.

Root surface PMEase activity of the bioassay seedlings ranged from 30 to 50 n *M* pNP g$^{-1}$ fwt s$^{-1}$ (Figure 2). The activity was significantly less in the N and N plus lime treatments than in the control and lime treatments. A significant, but weak, positive linear relationship was observed between root surface PMEase activity and shoot P concentration of the bioassay seedlings ($r2 = 28.7$, $P = 0.004$; PMEase = 21.7 × Shoot P + 1.17; Figure 3).

Soil microbial biomass carbon ($C_{mic}$) in the F and H horizons ranged from 153 to 241 $\mu$g g$^{-1}$ dwt (Table 3). These values were significantly lower in the lime and N plus lime treatments than in the control and N treatments. In the Ah horizon, $C_{mic}$ ranged from 50 to 79 $\mu$g g$^{-1}$ dwt and were not affected by any of the treatments. The metabolic quotient ($qCO_2$) in the F and H

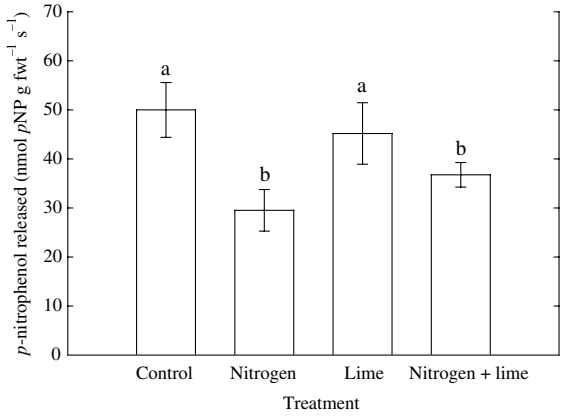


*Figure 2*. Root surface phosphomonoesterase activity in *A. capillaris* bioassay seedlings transplanted for 10 days into plots receiving factorial combinations of lime and N (±SE). Bars sharing a letter are not significantly different ($P > 0.05$; $n = 5$).

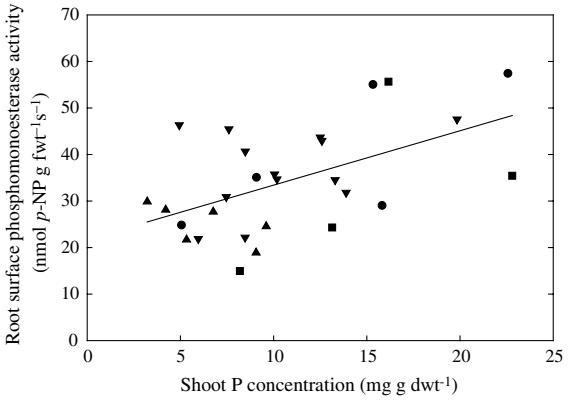


*Figure 3*. Relationship between root surface phosphomonoesterase activity and shoot P concentration of *A. capillaris* bioassay seedlings transplanted into intact grassland turfs removed from control plots (●) or plots receiving lime (■) and N (▲) or a combination of both (▼; $y = 1.17x + 21.7$, $R^2 = 28.7\%$, $P = 0.004$).

*Table 3*. Microbial biomass carbon ($C_{mic}$), basal respiration and metabolic quotient ($qCO_2$) in F and H and Ah horizons (±SE), and *P*-values derived from ANOVA of N and lime effects ($n = 5$)

| Treatment | F and H horizons | | | Ah horizon | | |
|---|---|---|---|---|---|---|
| | $C_{mic}$ ($\mu$g C g dwt$^{-1}$) | Basal respiration ($\mu$g $CO_2$ g dwt$^{-1}$) | $qCO_2$ | $C_{mic}$ ($\mu$g C g dwt$^{-1}$) | Basal ($\mu$g $CO_2$ g dwt$^{-1}$) | $qCO_2$ |
| Control | 241 ± 42.8 | 71.1 ± 10.3 | 0.31 ± 0.05 | 79 ± 22.3 | 21.7 ± 1.7 | 0.37 ± 0.09 |
| N | 205 ± 25.7 | 31.9 ± 3.6 | 0.17 ± 0.03 | 65 ± 14.4 | 15.7 ± 3.1 | 0.26 ± 0.04 |
| Lime | 162 ± 28.0 | 56.7 ± 5.0 | 0.38 ± 0.04 | 57 ± 7.4 | 27.1 ± 1.6 | 0.53 ± 0.12 |
| N plus lime | 153 ± 12.3 | 21.2 ± 4.4 | 0.14 ± 0.03 | 50 ± 11.1 | 13.0 ± 2.8 | 0.36 ± 0.15 |
| *P*-values from ANOVA | | | | | | |
| N | 0.649 | 0.000 | 0.000 | 0.419 | 0.000 | 0.165 |
| Lime | 0.038 | 0.036 | 0.695 | 0.251 | 0.895 | 0.241 |
| N plus lime | 0.669 | 0.368 | 0.246 | 0.950 | 0.172 | 0.696 |

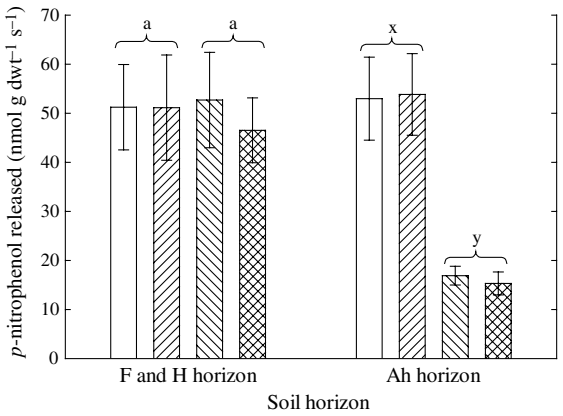


*Figure 4*. Soil phosphomonoesterase activity in response to liming and N fertilization (±SE). Control (□), N (▨), Lime (▧), N plus lime (▩). Bars sharing a letter are not significantly different ($P > 0.05$; $n = 5$).

horizons ranged from 0.14 to 0.38 (Table 3). The greatest values were in the control and lime treatments, these being double ($P < 0.05$) those in the N and N plus lime treatments. In the Ah horizon, $qCO_2$ ranged from 0.26 to 0.53, but in contrast to the F and H horizons, did not differ significantly between treatments.

Soil PMEase activity ranged from 46 to 53 n*M* g$^{-1}$ dwt s$^{-1}$ in the F and H horizons and from 15 to 54 n*M* g$^{-1}$ dwt s$^{-1}$ in the Ah horizon (Figure 4). In the F and H horizons no significant differences between the treatments were observed. In contrast, soil PMEase was approximately four times less in the lime and N plus lime treatments compared to the values in the control and N treatments.

## Discussion

The results demonstrate the sensitivity of the bioassay method for investigating changes in functional characteristics of plant roots and their associated AM fungi in long-term grasslands subjected to agricultural management regimes. The application of lime and N fertilizer for 2 years had a major effect on the soil microbial community of this upland grassland. Changes in both AM colonization and root-surface PMEase activity were measured after only 10-days growth of the bioassay seedlings. Both AM fungal colonization and root surface PMEase activity of *A. capillaris* seedlings increased, and a positive linear relationship was observed between the PMEase activity and shoot P concentration of the seedlings. This relationship provides good evidence that root surface PMEase activity can reflect, at least to some extent, the demand by plants for P.

Although the degree of AM fungal colonisation that we observed was less in the bioassay seedlings compared to levels found in mature plants from acid grassland (see e.g., Read et al., 1976), the speed by which seedling roots were colonised was comparable to a similar bioassay experiment undertaken in calcareous grassland using *P. lanceolata* (Read and Birch, 1988). In this experiment, the seedlings became colonised after only four days, while after 21 days heavy colonisation was observed. The rapidity of AM fungal colonisation highlights the importance for the plants to become quickly connected to the mycelial network (Van der Heijden, 2004). The

increase in AM fungal colonization of bioassay plant roots as a result of the liming is of particular significance as mycorrhizas have critical roles in uptake of plant-limiting nutrients, especially P. In the same grassland, plants with mycelial access to $^{33}$P-labelled phosphoric acid had shoot $^{33}$P concentrations an order of magnitude greater compared to plants with no mycelial access to the radioisotope (Johnson et al., 2001). Further experiments are required to test whether the increased early colonization of seedlings by AM fungi grown in the treated plots in the present experiment translates into greater tissue nutrient concentrations for the lifetime of the plants.

The root surface PMEase activity of the seedling bioassay plants showed no increase when they were grown under any of the treatment regimes. In a similar grassland, root surface PMEase activity of *A. capillaris* seedlings were not affected by 18 months of simulated atmospheric reactive N deposition (Johnson et al., 1999). In contrast, the authors found that application of the treatments for 5 years doubled their root surface PMEase activity (Johnson et al., 1999). It is clear therefore that plant P demand, as reflected by root surface PMEase activity, may lag considerably behind the application of acute fertilizer N as well as chronic deposition of atmospheric N, until the system is either sufficiently N saturated or the supply of plant available P has been depleted.

Soil PMEase activity was unaffected in the surface F and H horizons but decreased significantly in the Ah horizon in response to the application of lime, regardless of whether this was applied in combination with N. This is surprising since the most substantial increases in soil pH caused by the addition of lime occurred in the F and H horizons. Here, the pH increased by 2.5 units from 5.3 to 7.9, but in the Ah horizon increased by 1 unit from 4.9 to 5.9. Similar decreases in soil PMEase activities have been reported in other organic soils as a result of pH-induced increases as a consequence of lime applications (e.g. Antibus and Linkins, 1992). The data may also reflect increased resilience of microbes, a major contributor to total soil PMEase activities, to the liming treatments in the F and H horizons, or, reduced microbial demand for P. Orthophosphate has also been shown to be a potent repressor of PMEase activity (e.g. Kroehler and Linkins, 1988). However, the 20-fold difference in extractable soil P concentrations between the FH and Ah horizons, and the fact that soil PMEase activity (expressed on a gravimetric basis) did not show comparative differences between the horizons suggests that soil extractable P is not limiting in this system. Our results support those of Lovell et al. (1995) who reported that the application of 200 kg N ha$^{-1}$ for 5 years to a permanent pasture had no effect on PMEase activity measured in the upper 10 cm of the soil profile. In contrast, chronic inputs of N have been reported to significantly stimulate soil PMEase activity in both acid and calcareous grasslands (Johnson et al., 1998); the activity in the latter being strongly correlated to the concentration of KCl-extractable inorganic N in the soil.

Microbial biomass carbon was unaffected in the Ah horizon but was significantly decreased in plots receiving inputs of lime. However, the activity of the microbial biomass was strongly affected by addition of N, which caused a significant reduction in basal respiration rates from both the F and H and Ah horizons. Long-term N additions have been reported to reduce basal respiration in the Ah horizon of a similar acid brown earth, whereas no effects were observed in the shorter term (after 18 months; Johnson et al., 1998). Liming has for many years been recognised to cause substantial reductions in the quantity of carbon stored in grassland soils. The significant reduction in $C_{mic}$ may reflect changes in the input of labile carbon. Pulse-labelling experiments at Sourhope have shown that recent plant assimilate, which is likely to comprise readily available forms of carbon, is recycled much more quickly in limed plots than in control plots (Staddon et al., 2003).

The results reported here suggest that the soil microbial biomass, including both free-living and symbiotic organisms, is sensitive to short-term inputs of N and lime. In particular, they demonstrate that these treatments can have profound impacts on key components of the P cycle, notably the production of root-surface and soil PMEase activity and inoculum potential of mycorrhizal fungi.

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